Vaccinia Virus, Western Reserve Genome, VAC(WR)-LoxP-GFP-BAC/Zeo, Recombinant in *Escherichia coli*

Catalog No. NR-17606

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Contributor:

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Manufacturer:

BEI Resources

Product Description:

The entire vaccinia virus (VACV) Western Reserve (WR) genome (~195 Kb) (NCBI Accession AY243312) with a green fluorescent protein (GFP) sequence, two loxP sites, and a zeomycin resistance gene (*zeo*^{R}) was cloned as a bacterial artificial chromosome (BAC)[1] and grown in *Escherichia coli* (*E. coli*) DH10β cells harboring a mini-lambda prophage.[2] The integrated mini-lambda encodes the *red* recombination system, comprising a 5' to 3' exonuclease (Exo), a single-strand DNA binding protein (Bet), and a nuclease inhibitor (Gam), under the control of the temperature-sensitive λ cI857 repressor. *E. coli* DH10β/VAC-BAC/λ provides a system to achieve recombination between the VAC-BAC artificial chromosome and electroporated linear DNA with homologous sequences to facilitate production of recombinant VACV expression vectors or mutagenesis of the VACV genome.[2,3] The chloramphenicol acetyl transferase (*cat*) gene promoter in the VAC-BAC is inactivated by replacement with the *zeo*^{R} gene, making NR-17606 chloramphenicol sensitive and zeomycin resistant. Subsequent reactivation by inclusion of a *cat* promoter in the recombination cassette allows selection of recombinant VAC-BAC based on chloramphenicol resistance.

Material Provided:

Each vial contains approximately 200 µL of NR-17606 in Luria-Bertani (LB) broth supplemented with 10% glycerol.

Packaging/Storage:

NR-17606 was packaged aseptically in screw-capped plastic cryovials. The product is provided frozen on dry ice and should be stored at -60°C or colder immediately upon arrival. Freeze-thaw cycles should be minimized.

Growth:

Media: LB broth containing 25 µg/mL of zeomycin.
Incubation: 32°C in a shaking incubator at 200 rpm.

Functional Activity:

The presence of authentic VACV WR and GFP sequences in NR-17606 has been confirmed by PCR amplification and partial nucleotide sequencing. The sequence of the entire VACV WR genome has not been confirmed due to the large size of the plasmid insert. The presence of the integrated mini-lambda prophage also has not been confirmed at BEI Resources.

Citation:

Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: Vaccinia Virus, Western Reserve Genome, VAC(WR)-LoxP-GFP-BAC/Zeo, Recombinant in *Escherichia coli*, NR-17606."

Biosafety Level: 1

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. Biosafety in Microbiological and Biomedical Laboratories. 5th ed. Washington, DC: U.S. Government Printing Office, 2009; see www.cdc.gov/biosafety/publications/bmbl5/index.htm.

Disclaimers:

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NR-17606 is claimed in U.S. Patent Number 7,494,813 and the continuations, continuations-in-part, re-issues and foreign counterparts thereof. Commercial use requires a license from the U.S. Government. For further information contact the Office of Technology Transfer, National Institutes of Health, 6011 Executive Boulevard, Suite 325, Rockville, Maryland 20852-3804, (301) 496-7057.

References:

1. Domi, A. and B. Moss. "Cloning the Vaccinia Virus Genome as a Bacterial Artificial Chromosome in *Escherichia coli* and Recovery of Infectious Virus in Mammalian Cells." Proc. Natl. Acad. Sci. USA 99 (2002) 12415-12420. PubMed: 12196634.
2. Domi, A. and B. Moss. "Engineering of a Vaccinia Virus Bacterial Artificial Chromosome in *Escherichia coli* by Bacteriophage Lambda-Based Recombination." Nat. Methods 2 (2005): 95–97. PubMed: 15782205.
3. Court, D.L., et al. "Mini-Lambda: A Tractable System for Chromosome and BAC Engineering." Gene 315 (2003): 63-69. Pubmed: 14557065.

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